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Preliminary Investigation of Bovine Respiratory Disease Complex in Indonesia

S. Setiyaningsih[1]*, M. Shofa[1], R. Wulansari[1], B. P. Priosoeryanto[1]

[1]Faculty of Veterinary Medicine, Bogor Agricultural University (IPB), Kampus IPB Dramaga 16680 Bogor, Indonesia
*Corresponding author email: surachmi@apps.ipb.ac.id

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INTRODUCTION

Bovine Respiratory Disease Complex (BRDC) has been recognized as a major cause of cattle morbidity and mortality throughout the world, which greatly affects beef and dairy cattle industries. It develops as a result of complex interactions between environment, cattle, and pathogens. Multiple viral or bacterial agents have been documented in BRDC cases. These include Bovine Herpesvirus 1 (BoHV-1), Bovine Viral Diarrheal Virus (BVDV), and Bovine Parainfluenza Virus-3 (BPIV-3), Bovine Respiratory Syncytial Virus (BRSV); Bovine Coronavirus, Bovine influenza D Virus, Bovine Rhinitis A and B viruses, Bovine Adenovirus, *Mannheimia haemolytica* (MH), *Pasteurella multocida* (PM), *Histophilus somni* (HS), *Klebsiella pnemoniae*, *Truperella pyogenes*, *Ureaplasma diversum*, and *Mycoplasma bovis* (MB) (1). Indonesia imports considerable number of life beef and dairy cattle from Australia and New Zealand. Close proximity of cattle in a high-density during transportation and stress related to dramatic environmental changes often give rise to significant losses due to respiratory diseases. Accordingly, feedlot and dairy cattle in Indonesia are likely to have high risk of experiencing BRDC. This preliminary study was aimed to investigate the occurrence of BRDC and to identify its associated pathogens in beef and dairy cattle in limited area of western Java.

MATERIALS AND METHODS

Sample. Fifty-two cattle, 32 Holstein-Friesian and 20 Brahman cross, were included in this preliminary study. A total of 69 respiratory samples were collected; distal tracheal swab and lung tissue were taken from Bogor and Tangerang. Clinical and pathological examinations were carried out whenever appropriate. Each respiratory sample was collected into 3 ml of PBS. Samples were transported in a cool box with ice packs and were stored at -20°C until further use.

PCR Assay. Total RNA and DNA were extracted from respiratory swab samples using Nucleospin (TaKaRa) and from lung tissue samples using DNeasy Blood & Tissue Kits (Qiagen) following the provided protocols. Extracted RNA and DNA were stored at –25ºC until needed. Amplifications were performed with Taq PCR Kit (QIAGEN) and RT-PCR using One-Step RT-PCR kit (QIAGEN). Panels of pathogen specific primers were used to detect eight viral and bacterial pathogens, i.e. BoHV 1, BRSV, BVDV, BPI-3, PM, MH, HS, and MB. The amplifications were done according to the recommended protocols (2). Amplified DNA was analyzed by agarose gel electrophoresis and visualized using UV transilluminator.

RESULT AND DISCUSSION

Majority of cattle in this study demonstrated respiratory signs and lesions; only 2 dairy cows were apparently healthy. Gross visible lesions were observed in the lungs of all 17 slaughtered cows while respiratory signs were evidenced in all 3 feedlot and 30 dairy cows. The presence of selected viral and bacterial pathogens is summarized in Table 1. Overall, four different BRDC potential pathogens were detected in a total of 20/52 (38.5%) cattle. The most frequently identified pathogen was PM (36.5%; 19/52), followed by MB (5.8%; 3/52), BVDV (3.8%; 2/52) and BoHV-1 (1.9%; 1/52). All of those four different pathogens were detected in dairy (53.1%; 17/32), whereas only PM was present in beef cattle (15.0%; 3/20).

Fifteen cows carried only a single respiratory disease pathogen; majority of cows (11 dairy and 3 beef) had PM only, whereas just one dairy cow had BVDV only. One of these PM-positive dairy cows was apparently healthy. Presence of multiple respiratory pathogens was identified in five dairy cows, with combinations of PM and MB (3 cows), PM and BVDV, and PM and BoHV-1 in one cow each, while MH, HS, BRSV, and BPI-3 were not detected. No potential respiratory pathogen was detected in 31 cattle (59.6%) although respiratory signs and lung lesions were noticeable.

MH, PM and MB are the most important bacteria involved in BRDC cases worldwide. In our study, PM was the most prevalent respiratory

pathogen, which predominantly identified as a single infection. Although it has been generally accepted that PM is a commensal bacteria of upper respiratory tract, the ability of PM to cause cell injury by inducing various inflammatory cytokines has been demonstrated (3,4). Nevertheless, the role of PM either as a primary or simply opportunistic pathogen in BRDC is still being debated. Further study to explore characteristic determinant that is capable of discriminating between commensal and pathogenic PM strains is essential.

Microbes	Dairy Cattle (n=32)		Beef Cattle (n=20)		Total (n=52)	
	Number	%	Number	%	Number	%
PM	11	34,4	3	15,0	14	26,9
PM,MB	3	9,4	0	0,0	3	5,8
BVDV	1	3,1	0	0,0	1	1,9
PM, BVDV	1	3,1	0	0,0	1	1,9
PM, BoHV	1	3,1	0	0,0	1	1,9
Total	**17**	**53,1**	**3**	**15,0**	**20**	**38,5**

Table 1. BRDC related gene. Number and percentage of selected BRDC important pathogens identified by PCR. Other pathogens, MH, HS, BRSV, and BPI-3, were not detected.

A number of studies substantiate the prominent role of initial viral infection in BRDC pathogenesis specifically in promoting bacterial colonization. Similar role is also suggested for mycoplasma. Here, we showed dual infection with MB and PM in three dairy cows; similar findings were also documented in other countries (2,5). Furthermore, our study identified two important BRDC viral pathogens in other three dairy cows that housed in the same facility; two cows had BVDV and the other had BoHV-1. One lactating cow had BVDV only, and one calves harbored BVDV and PM. The other lactating cow harboured BoHV-1 and PM. Tropism of BVDV for lymphoid cells has been recognized to cause immune suppression, thus increasing the risk of secondary bacterial infection (5). Similar fashion also reported for BoHV-1, which productive infections can be triggered by immunosupression or epithelial cell injury due to coinfecting PM [6]. Evidence of synergistic interactions between pathogens causing BRDC has been established; nevertheless, viral and bacterial infections alone have also been documented in BRDC cases (3,4,5,6). Our findings confirm the involvement bacterial and viral pathogens, either as single or multiple infections, which may contribute to the respiratory disease in dairy and beef cattle. Further exploration using more sensitive techniques to detect more diverse array of pathogens in larger number of cattle is warranted.

CONCLUSIONS

Our study showed that PM was the most prevalent pathogen detected in BRDC cases, mainly identified alone or occasionally in combination with bacteria or viruses. The absence of BRDC potential pathogens in a reasonably high proportion of cattle despite noticeable respiratory symptoms and lesions highlights the need for more sensitive detection techniques and the possibility of the involvement of other microbial pathogens in BRDC cases in Indonesia.

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